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For research use only

Cat. No. INT-D30210 | **96 Tests**

VeTeK™ ILTV Detection Kit

Test for the detection of Infectious Laryngotracheitis Virus by one-step PCR

User Manual

REV.2.2

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1. DESCRIPTION

Infectious laryngotracheitis virus (ILTV) causes a serious, worldwide-occurring respiratory disease of chickens which affects growth and egg production and may lead to death of the animals. The acute phase of infection lasts between 1 and 2 weeks and is often associated with clinical signs like gasping, coughing, expectoration of bloody mucus, and conjunctivitis. Subsequently, an asymptomatic latent infection of the central nervous system can be established.

For prevention of disease chickens are immunized with attenuated live-virus vaccines that are suitable for mass application via eye drop, aerosol, or drinking water. Infectious laryngotracheitis is a contagious respiratory disease which is characterized by gasping, neck extension and conjunctivitis (inflammation of the membrane around the eye).

ILTV, also designated as gallid herpes virus 1, has been classified as a member of the *Alphaherpesvirinae* subfamily of the *Herpesviridae* and represents the only species presently in the genus Infectious Laryngotracheitis-like Viruses.

VeTeK™ ILTV Detection Kit is direct detection of infectious laryngotracheitis virus on the basis of a genetic database, so it can diagnose very fast and accurately. It can amplify only specific gene using the PCR (Polymerase Chain Reaction) method, and take only 2~3 hours for detection. Therefore, it is a very fast accurate, reliable technique.

2. STORAGE

The components of VeTeK™ ILTV Detection Kit should be stored at -20℃, under this condition, the kit is stable until expiration date stated on the label.

3. CONTENTS

VeTeK™ ILTV PCR Pre-mixture ····· 96 tubes
DNase/RNase-free water (white cap) ····· 1 vial
ILTV positive control (Yellow cap) ····· 1 vial

Component in 20㎕ reaction
i-StarTaq™ DNA Polymerase
dNTPs
10X Reaction buffer
Chemical stabilizer
Gel loading buffer
8-MOP (dissolved in DMSO)
Primers for ILTV

4. SPECIMEN

Performs the test with whole blood, tissue or cerebrospinal fluid. The specimen should be stored at -20℃ prior to use.

5. ADDITIONAL REQUIRED MATERIALS

- Disposable gloves
- DNA extraction kit (see 6.1 DNA preparation method)
- Pipettes
- Sterile pipette tip
- Vortex mixer
- Centrifuge for microcentrifuge tubes
- Thermal cycler
- Electrophoresis kit
- UV transilluminator

6. PROCEDURE

Please read through the entire procedure before starting.

6.1 DNA Preparation

Various manufacturers offer DNA isolation kits. Please carry out the DNA isolation according to the manufacturer's instructions. The following standard extraction kit is recommended.

Product	Catalog No.	Manufacturer
Viral gene-spin™ Viral DNA/RNA Extraction Kit	17151	iNtRON Biotechnology, Inc.

6.2 Amplification

① Prepare appropriate PCR premix tubes and label. And one PCR premix tube for positive control.
② Add 2㎕ of template DNA into the PCR premix tube.
③ Add 18㎕ of DNase/RNase-free water into the PCR premix tube to total volume as 20㎕.
④ Add 2㎕ of positive control and 18㎕ of RNase-free water into a PCR premix tube for monitoring of amplification and easy interpretation.
⑤ Dissolve the blue pellet by pipetting.
Note :The pellet is easily dissolved, by letting the mixture stand at R.T. for 1-2minutes after adding water.
⑥ (*Optional)* Add mineral oil. This step is unnecessary when using a thermal cycler that employs a top heating method (general methods).
⑦ Perform PCR reaction of samples as the below process using PCR machine.

PCR cycle		Temp.	Time
1 Cycle	Initial Denaturation	94℃	5 min.
40 Cycles	Denaturation	94℃	30 sec.
	Annealing	52℃	30 sec.
	Extension	72℃	40 sec.
1 Cycle	Final extension	72℃	5 min.

6.3 Detection of Amplified Products

① Prepare 1.5% agarose gel containing Ethidium bromide (Et-Br).

② Load 7㎕ of PCR product and positive control on agarose gel without adding a loading-dye buffer and perform electrophoresis.

③ Run electrophoresis by 100V (required about 30~40 minutes).

④ Identify the result on ultra-violet (UV) transilluminator.

6.4 Interpretation

- Expected PCR product size : 477 bp

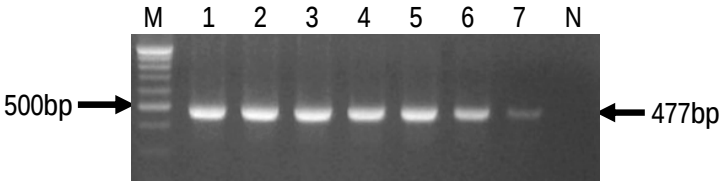

Fig 1. Electrophoresis of PCR product by VeTeK™ ILTV Detection Kit
Lane M : 100bp Molecular ladder (iNtRON Biotechnology)
Lane 1~7 : ILTV positive sample
Lane N : Negative control

6.5 Elimination of carry-over contamination

- Each PCR/RT-PCR Pre-mixture contains 8-methoxypsoralen (8-MOP) for preventing of carry-over contamination.
- All PCR products should be discarded after UV irradiation (10 min/365nm) for preventing from carry-over contamination.

7. NOTICE

- For research purpose only. Not for use in diagnostic procedures for clinical purposes. *For in Vitro Use Only*.
- Do not use any reagent after the expiration date.
- Do not use together with reagents of other products.
- Follow the instructions.
- Take care in handling of specimen to minimize risk of infection.
- The PCR process is covered by patents issued and applicable in certain countries. iNtRON Biotechnology, Inc. does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

8. TROUBLE SHOOTING

① In the case of difficult to interpret results due to non-specific bands.
☞ Reduce amount of template by 1/10 dilution and reacts again.

② Preparation of PCR reaction at room temperature may cause the non-specific band.

③ All procedure should be carried out on ice.

9. ORDERING INFORMATION

Product	Catalog No.
VeTeK™ ILTV Detection Kit	D30210
Viral Gene-spin™ Viral DNA/RNA Extraction kit	17151
100bp Ladder Molecular Weight DNA Marker	24012

Li StarFish distribuisce: